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Original article

ANTIMICROBIAL ACTIVITIES OF *GOSSYPIUM HIRSUTUM* (COTTON) AND *ABELMOSCHUS ESCULENTUS* (OKRA) LEAVES EXTRACTS

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Abstract

Background: This study evaluates the antimicrobial activities, phytochemical composition, and thin layer chromatography (TLC) profile of ethanolic and aqueous leaf extracts of *Gossypium hirsutum* L. and *Abelmoschus esculentus (L.) Moench*.

Aims: The objective is to investigate the phytochemical constituents and assess the antimicrobial efficacy of these plant extracts against various pathogenic microorganisms.

Methods: Leaves were collected, identified, air-dried, and powdered. Ethanolic and aqueous extracts were prepared and subjected to TLC analysis using silica gel plates and a mobile phase of ethanol, methanol, and ethyl acetate (5:3:2). Phytochemical screening was conducted using standard protocols. Antimicrobial activity was evaluated using the agar well diffusion method against pathogens including *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Salmonella typhi, Proteus, Streptococcus pneumoniae* and *Candida spp*.

Results: TLC analysis revealed seven compounds in *G. hirsutum* and six in *Abelmoschus esculentus* with distinct Rf values. Phytochemical screening identified phenols/tannins, saponins, flavonoids, reducing sugars, terpenoids, and anthraquinones in *G. hirsutum*, while *A. esculentus* contained all tested phytochemicals except phytosterol and essential oil. The antimicrobial assay indicated that ethanolic extracts of both plants exhibited significant antibacterial activity, with *G. hirsutum* showing higher efficacy. Ethanolic extracts of *G. hirsutum* inhibited *S. aureus* and *S. typhi* effectively, while aqueous extracts showed limited activity. Extracts of *A. esculentus* leaves inhibited all tested organisms except Proteus, with ethanol extracts also ineffective against *S. pneumoniae*.

Conclusion: The study demonstrates that ethanolic extracts of *G. hirsutum* and *A. esculentus* possess antimicrobial properties. These findings support the potential use of these plant extracts in developing natural antimicrobial agents.

1. INTRODUCTION

The resistance of the bacteria to the innumerous antimicrobial agents constitutes one of the great challenges in the treatment of infections (Nunes et al., 2006). Antimicrobial resistance is a global problem that impacts all countries and all people regardless of their wealth or status. The rise and spread of antimicrobial resistance is creating a new generation of superbugs that cannot be treated with existing medicines activity (Vijapur et al., 2021). The cause of the occurrence of resistance to antimicrobial agents is mainly associated with the misuse of the drugs (Saddiq et al., 2023).

Plants are recognized as the primary source in traditional care systems due to their curative potency (Begum et al., 2020). Traditional herbal medicine development and utilization can be traced back to the Stone Age. Traditional healing and magic or incantations appear to be significantly more common than Western medicine (Bunu et al., 2023). A medicinal plant is any plant material be it seeds, root extracts or leaves extract that is used to cure or fight against infection or attempt to maintain health, which are to be administered for specific ailments which can either be in modern or traditional medicine (Lawal et al., 2019). This is because plants are naturally endowed with phytochemicals capable of curing a number of diseases (Saddiq et al,. 2023).

Gossypium L. is one of the genera under the leaf succulent family malvaceae (Nwobodo, 2013; Al-Snafi, 2018). It is generally referred to as cotton plants are used indiscriminately in traditional medicine without cognizance to the fact that there are various cotton species and the likelihood of differences in phytochemical content and importantly, their medicinal capabilities (Oni et al., 2022). It is a perennial shrub that grows to approximately 1.5- 2 m and has the potential to develop leaves, stem, flowers, fruits and seeds all at the same time. The seed vary from black and smooth to green with tightly adhering fuzz (Ayeni et al., 2015) and germinates in 14-21 days at 20-23˚C (Chinweuba et al., 2013). The name “cotton” (English) originated from the Arabic term “al qutn” and it describes species that produce spinnable fibres (lint) on their seed coat. The name 'cotton plant' is actually used for four species in the genus *Gossypium; G. hirsutum* L., *G. barbadense* L., *G. arboretum* L. and *G. herbaceum* L. that were domesticated independently as a source of textile fiber (Ade-Ademilua and Okpoma, 2018).

The plant *A. esculentus* (L.) Moench, formally known as *Hibiscus esculentus* L (Carla et al., 2011). *A. esculentus* (Okra) is belonging to the family Malvaceae (Chancha et al., 2018), it is an important vegetable crop grown mainly in the tropical or sub-tropical regions during summer and rainy season (Solomon et al., 2016). It is principally used in the preparation of soup in Nigeria (Chukwuka et al., 2014).It is apparently originated in Ethiopia, higher parts of the Anglo-Egyptian Sudan (Binalfew & Alemu, 2016), Okra, commonly called gumbo, ockro, okro and bhindi (Sandra, 2017) and known as “lady finger” (Binalfew & Alemu, 2016; Chaudhari et al., 2011). Traditionally parts of the plants are assumed to have medicinal properties like antioxidant antispasmodic, demulcent, diaphoretic, diuretic, emollient, and stimulant (Chaudhari et al., 2011).

While various research studies have explored different species of the genera *Gossypium* L. and *Abelmoschus* Medik., scientific findings on the pharmacological activities of the plant found in Askira Uba, Borno State, Nigeria remain limited. The aim of this research is to compare the therapeutic properties of *G. hirsutum* and *A. esculentus* with established therapeutic data of species of the plants’ genera from the literature.

2 Materials and Methods

2.1 Chemicals and Reagents

Unless otherwise stated all chemicals and reagents used were of analytical grade and purchased from Scientific Laboratory (Jimeta-Yola, Adamawa State, Nigeria).

2.2 Sample Collection and Identification

Gossypium hirsutum L. and *Abelmoschus esculentus (L.) Moench* leaves were collected from the bush area of Askira Uba, Local Government Area of Borno State, Nigeria, and were identified by Professor Dimas Kubmarawa, a Professor of Natural Products in the Department of Chemistry, Modibbo Adama University, Yola.

2.3 Preparation of Sample

The leaves of *G. hirsutum* and *A. esculentus* plants were washed and air-dried in Chemistry Laboratory 2, Science Complex of the Faculty of Science, Adamawa State University, Mubi, under shade at room temperature. They were then weighed and ground to a coarse powder using a sterile mortar and pestle. The powders were stored in an airtight container and used for successive analysis (Kubmarawa et al., 2009).

2.4 Plant Preparation and Extraction

100 g of the *G. hirsutum* and *A. esculentus* powder were weighed and extracted using ethanol and water solvents in separate airtight containers for 24 hours. The resultant mixtures were filtered with filter paper (Whatman No. 1) under gravity. The filtrates were dried at 60°C on a water bath to yield ethanolic extract residue (Adamu et al., 2018).

2.5 Thin Layer Chromatography (TLC) Analyses

Thin layer chromatography was carried out on the ethanolic extract using silica gel pre-coated glass plates. Two grams of concentrated dry extract of *G. hirsutum* and *A. esculentus* samples were diluted with 70% ethanol. Samples were adjusted to a volume of 1 mL, which was further spotted on preparative TLC plates (Giri et al., 2020). A microsyringe was used to uniformly apply 10 µL of the extracts on the TLC plate to make a spot, which was then allowed to dry. The plates were developed in a chromatographic tank using a mobile phase comprising ethanol, methanol, and ethyl acetate (5:3:2). The developed plates were air-dried. The retardation factor (Rf) for each component was calculated using the following formula:

$$\boldsymbol{Rf} = \frac{\boldsymbol{Distance\ travelled\ by\ compound\ (DC)}}{\boldsymbol{Distance\ travelled\ by\ solvent\ front\ (DS)}}$$ (Kumar *et al.,* 2013).

2.6 Phytochemical Screening

Phytochemical screening was performed using standard procedures. By using different specific reagents, the presence of main groups of natural products was detected in the ethanolic extracts of *G. hirsutum* and *A. esculentus*.

2.6.1 Alkaloids

To a few milliliters of the plant sample extract, two drops of Mayer's reagent are added along the sides of the test tube. The appearance of a white creamy precipitate indicates the presence of alkaloids (Banu and Catherine, 2015).

2.6.2 Phenolic Compounds and tannins

50 mg of the extract is dissolved in 5 mL of distilled water. To this, a few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compounds. For the detection of tannins, 2 mL of the extract is placed in a test tube and gently heated for 2 minutes. An orange color observed after adding 3 drops of ferric chloride indicates the presence of tannins (Rashed et al., 2019).

2.6.3 Saponins

5 mL of the extract sample was diluted with 15 mL of distilled water. The resultant mixture was shaken strongly; the appearance of foam indicates the presence of saponins (Oscar et al., 2020).

2.6.4 Flavonoids

A portion of the extract was dissolved in 2 mL of 50% methanol. Metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. The appearance of a red color indicates the presence of flavonoids (Namadina et al., 2020).

2.6.5 Glycosides

Glycosides were detected by adding a few drops of glacial acetic acid, ferric chloride, and concentrated sulfuric acid to each sample through the side wall of the test tube. A reddish-brown color appeared at the junction of the two layers, and the upper layer appeared bluish-green (Rajitha et al., 2022).

2.6.6 Phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of concentrated sulfuric acid, shaken, and allowed to stand. The appearance of a golden yellow color indicates the presence of triterpenes (Khanal et al., 2015).

2.6.7 Reducing Sugars

The aqueous ethanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a color reaction (Ayoola et al., 2008).

2.6.8 Proteins and Amino Acids

Xanthoproteic Test: The extracts were treated with a few drops of concentrated nitric acid solution. The formation of a yellow color indicates the presence of proteins (Khanal et al., 2015).

2.6.9 Terpenoids

A small sample of the extract was treated with 1 mL of acetic anhydride, 1 mL of trichloromethane, and 1 mL of sulfuric acid. The production of a violet color indicates the presence of terpenoids (Oscar et al., 2020).

2.6.10 Anthraquinones

To a portion of the extract in a dry test tube, 5 mL of chloroform was added and shaken for at least 5 minutes. This was filtered, and the filtrate was shaken with an equal volume of 10 % ammonium solution. A bright pink color in the aqueous upper layer indicates the presence of free anthraquinones (Namadina et al., 2020).

2.6.11 Essential Oils

Two drops of ferric chloride were added to 90% alcohol containing a small quantity of the extracts. The appearance of a greenish coloration indicated the presence of essential oils (Muhammad et al., 2018).

2.7 Antimicrobial Test

2.7.1 Collection of Microorganisms

The test organisms used were collected from the stock cultures of the General Hospital, Infinity Medical Laboratory, Ecogate Clinic and Maternity, and New Life Hospital, all located in Mubi Local Government Area of Adamawa State, Nigeria. The microorganisms are: *E. coli*, *S. aureus*, *K. pneumoniae*, *S. typhi, P. vulgaris*, *S. pneumoniae*, and *Candida.*

2.7.2 Solvent Extraction

A quantity of 200 g of plant powder was extracted in 200 mL of ethanol (95 % w/v) for 24 hours, strained, and the extract was concentrated to dryness at 60 °C in a water bath. The same procedure was used for aqueous extraction. The extracts were then refrigerated at 4 °C prior to use. Amounts of 3 grams, 5 grams, 7 grams, and 9 grams of the extract were reconstituted in 10 mL of sterile distilled water to obtain solutions of 0.3, 0.5, 0.7, and 0.9 g/mL concentrations, respectively while the reference drug, ciprofloxacin USP 500 mg prepared at 0,9, 0.7, 0.5, 0.3 and 0.1 mg/mL, respectively were used for the antimicrobial screening (Ogunsola and Fasola, 2014).

2.7.3 Preparation of Media

Two grams of nutrient agar was homogenized in 1 liter of distilled water using a water bath at 100°C. This was then autoclaved at 121 °C for 15 minutes. The medium was cooled to 45 °C after autoclaving before pouring into plates and used for subsequent bacteria plating (Ogunsola and Fasola, 2014).

2.7.4 Evaluation of Antimicrobial Test

The level of susceptibility of each test organism was determined using the agar well diffusion method. The plates were inoculated with the test isolates. Afterwards, a sterile cork borer of 5 mm diameter was used to make holes in the nutrient agar plates. 0.2 mL of the extract was filled into each appropriately labeled well. The inoculated plates were kept at room temperature for 30 minutes to allow the extract to diffuse into the agar and were then incubated at 37 °C for 24 hours. Antimicrobial activity was determined by zones of inhibition, which were quantified by measuring the diameter of the zone of inhibition in millimeters (mm) using a meter rule after incubation (Okorondu et al., 2015).

3 RESULTS AND DISCUSSION

3.1 Preliminary Thin Layer Chromatography Profiling

The TLC of the ethanol extract of *G. hirsutum* leaves revealed the presence of seven (7) compounds while the ethanol extract of *A. esculentus* leaves revealed the presence of six (6) compounds as shown in Figure 1. The distance moved by the solvent (DS) is 12 cm. The presence of different Rf values shows that there is a distinction between diverse components isolated individual representatives implying that each plant has several phytochemicals. Comparative between *G. hirsutum* and *A. esculentus* with greater Rf value spread in *G. hirsutum* may suggest possibilities for a higher chemicals diversity in *G. hirsutum*.

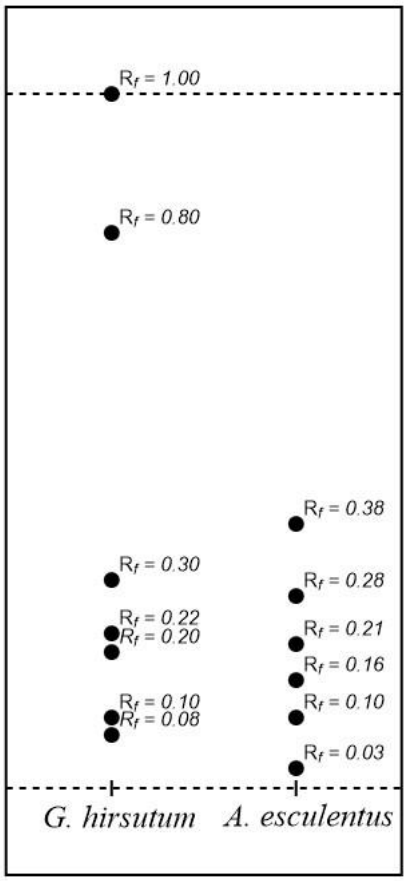

Figure 1. TLC sketch of the thin layer chromatography of *Gossypium hirsutum* and *Abelmoschus esculentus* ethanolic extracts (created with ChemDraw).

3.2 Phytochemical Screening of *G. hirsutum* and *A. esculentus*

The preliminary phytochemical screening conducted on the ethanol extracts of *G. hirsutum* and *A. esculentus* leaves revealed the presence of various bioactive secondary metabolites, confirmed through chemical color reaction tests, as shown in Table 1. The results from the phytochemical analysis of *G. hirsutum* leaf extracts showed the presence of phenols/tannins, saponins, flavonoids, reducing sugars, terpenoids, and anthraquinones, while alkaloids, glycosides, and proteins/amino acids were found to be absent. For *A. esculentus*, all the phytochemicals tested were present except for phytosterols and essential oils, which were absent in both plant extracts. These findings are consistent with those of Abdel-Razek et al. (2023), who reported that flavonoids and terpenes are the main secondary metabolites detected in all parts of *A. esculentus*. Ayushi et al. (2016) reported that the aqueous extract of *A. esculentus* leaves showed a significant presence of alkaloids, carbohydrates, flavonoids, terpenoids, and tannins compared to saponins, steroids, and glycosides, which were present to a lesser extent. They noted that anthocyanins, anthraquinones, and proteins were completely absent.

In contrast, Al-Snafi (2018) and Patil et al. (2014) found that *G. hirsutum* contained alkaloids, phenolic compounds, terpenoids, tannins, saponins, flavonoids, cardiac glycosides, and proteins. The phytochemical analysis conducted in this study indicated that *G. hirsutum* leaves also contained bioactive compounds, which are the primary basis for the medicinal properties of the plant. Ayeni et al. reported the presence of and medicinal properties of secondary plant metabolites such as alkaloids, saponins, cardiac glycosides, flavonoids and tannins in the leaves of *G. hirsutum* and *M. charantia* as reason why they are used as medicinal plants (Ayeni et al., 2015).

Phytochemicals determined in *G. herbaceum* leaf extract, a species of the genus *Gossypium*, include tannins, anthraquinones, flavonoids, and terpenoids, which are similar to the findings of this study except for the absence of cardiac glycosides, which were not present in the *Gossypium* species used in this research (Adekanmbi et al., 2024). Ade-Ademilua and Okpoma (2018) also confirmed the presence of tannins in *G. hirsutum* leaf extract.

Table 1. Phytochemical Composition of *G. hirsutum* and *A. esculentus* etanolic extracts

S/N	Phytoconstituent	*G. hirsutum* Extract	*A. Esculentus* Extract
1	Alkaloids	-	+
2	Tannins	+	+
3	Saponins	+	+
4	Flavonoids	+	+
5	Glycosides	-	+
6	Phytosterols	-	-
7	Reducing Sugars	+	+
8	Proteins	-	+
9	Terpenoids	+	+
10	Anthraquinones	+	+
11	Essential Oil	-	-

***Key: (*+) present, (-) absent**

3.3 Antimicrobial Tests

3.3.1 Antimicrobial Inhibition of G. hirsutum extracts on test organisms

The pharmacological action of a plant cannot be ascertained by phytochemical studies alone, highlighting the need for antimicrobial tests (Ade-Ademilua and Okpoma, 2018). Table 2 shows the diameter of the zone of inhibition (mm) of various pathogenic organisms tested with the ethanol and aqueous leaf extracts of *G. hirsutum* and *A. esculentus*.

The antimicrobial assay confirmed that the leaf extracts exhibited significant antibiotic activity compared to the reference drug, Ciprofloxacin USP 500 mg, against some of the bacteria tested. The results of this study indicated that the ethanol extract of *G. hirsutum* was more effective against test pathogenic organisms compared to its aqueous extract in a concentration-dependent manner. No inhibition was observed with Proteus for either extract. The ethanol extract demonstrated inhibition against *S. typhi,* whereas the aqueous extract showed no inhibition.

Ade-Ademilua and Okpoma (2018) found antimicrobial activity of ethanol leaf extract of *G. hirsutum* against *E. coli* (11.33 mm), *S. aureus* (11.33 mm), *S. sonnei* (8.67 mm), *P. aeruginosa* (11.00 mm), *E. coli* ATCC 25922 (9.67 mm), and *K. pneumoniae* (10.00 mm) at 0.5 g/mL. Egbuta et al. (2017) noted that compounds derived from cotton have exhibited antimicrobial activity against fungi and bacteria in both in vitro and in vivo studies at concentrations between 5 µg/mL and 117 µg/mL. Al-Snafi (2018) observed that the free flavonoid fraction of *G. hirsutum* seeds' edible oil demonstrated antibacterial and antifungal activity with a diameter of inhibition of 12.33, 10, 10.16 mm against *E. coli*, *T. rubrum*, and *C. albicans* respectively at a concentration of 1 mg/mL.

Saddiq et al. (2023) reported the Mean Zone of Inhibition of another species of the genus, *Gossypium*, with 28.50, 26.40, and 21.00 mm against *E. coli*, *P. aeruginosa*, and *S. aureus* respectively at the highest concentration of 500 mg/mL of *G. barbadense* leaves ethanol fraction. Contrary to this, Chaturvedi et al. (2009) reported antimicrobial activity of free flavonoids (FF) and bound flavonoids (BF) fractions from cotton seed of *G. hirsutum* with the FF fraction showing activity index of 0.35 against *E. coli* and the BF with activity index of 0.32 against *S. aureus* and no activity against *P. aeruginosa*, while both fractions of the tissue culture (Callus) show no microbial resistance against the three organisms earlier mentioned. On the other hand, Vijapur et al. (2021) found that *G. hirsutum* ethanolic and aqueous leaf extracts did not show any inhibition against *S. aureus*, *S. mutans, K. pneumoniae, P. aeruginosa, C. albicans,* and *A. niger* at a concentration of 2mM but demonstrate appreciable activity with ethanol and aqueous silver nanoparticles of *G. hirsutum* at a concentration of 10 mg/mL. Lima et al. (2021) observed that *G. hirsutum* alcoholic leaf extracts exhibited inhibitory activity against *S. aureus*, with promising results from the 30% alcoholic extract, and were capable of inhibiting the growth of *E. coli* strains. Differently, the acetone leaf extract of *G. herbaceum* was active against *E. coli* (MIC 6.3 mg/mL), and other species of bacteria as *E. faecalis* (MIC 1.6 mg/mL), *P. aeruginosa* (MIC 3.1 mg/mL), and *S. aureus* (MIC 0.78 mg/mL).

Ikobi et al. (2012) also reported on a different species of *Gossypium* that methanol extracts of *G. barbadense* leaves demonstrated antibacterial activities against four of the five test organisms (*P. aeruginosa*, *S. aureus*, *E. coli*, *P. mirabilis*, and *S. sonnei*) with mean inhibition zone diameters (IZD) ranging from 12 to 17 mm. At a concentration of 10 mg/mL, the extract did not inhibit *P. mirabilis* and *S. sonnei*, but inhibited the growth of these two organisms at 20 and 30 mg/mL, while the extract shows no inhibition activity at all against *E. coli* at all concentrations. Similarly, El-Mesallamy et al. (2023) found that methanolic leaf extracts of *G. barbadense* L. exhibited antibacterial activity against *S. aureus* and *E. coli* only out of the six microorganisms used with inhibition zones of 10 mm and 15 mm, respectively, and showed no inhibition against *P. aeruginosa*, *S. enterica, A. niger,* and *A. fumigatus*. The result also revealed that the bolls and flower extract demonstrated higher antimicrobial activity than the leaf extracts in terms of their diameter of inhibition zone. Similarly, Anyanwu and Okoye (2017) and Mustapha et al. (2015) reported that *A. occidentale* and *G. hirsutum*, traditionally used to treat stomach ailments in Southwest Nigeria, had minimum inhibitory concentrations (MIC) ranging from 0.05 to 0.10 mg/mL of their ethanol leaf extracts against *S. aureus*, *E. coli*, *S. dysenteriae*, *S. typhimurium,* and *P. aeruginosa*.

Adekanmbi et al. (2024) observed that *G. herbaceum* methanol leaf extract demonstrated antibacterial effect against various test organisms obtained from various sources at varying concentrations: *S. aureus* (200 mg/mL, urine), *S. faecalis* (25 mg/mL, semen), *S. pyogenes* (6.25 mg/mL, High Vaginal Swabs, HVS), *K. pneumoniae* (100 mg/mL, urine), *P. mirabilis* (200 mg/mL, urine), *P. aeruginosa* (50 mg/mL, semen) and *E. coli* (6.25 mg/mL, urine). Islam et al. (2007-2010) found that ethanol whole plant extracts of *M. parviflora* L., a member of the Malvaceae family along with *G. hirsutum*, displayed antibacterial activity at 5, 50, and 100 mg/mL against *B. subtilis* with inhibition diameters ranging from 9.67 to 12.87 mm.

The findings suggest that bioactive compounds in these plant leaves could be incorporated into the synthesis of new drugs (Chinweuba et al., 2013).

Table 2. Antimicrobial Inhibition of *G. hirsutum* aqueous and ethanolic extracts

Treatment	Zone Of Inhibition (mm)					
	Aqueous extract			Ethanolic extract		
Extract Concentration (g/mL)	**0.9**	**0.7**	**0.5**	**0.9**	**0.7**	**0.5**
P. vulgaris (g/mL)	-	-	-	-	-	-
K. pneumoniae (g/mL)	10	-	-	12	8	-
S. aureus (g/mL)	14	9	-	13	10	7
S. typhi (g/mL)	-	-	-	14	-	-
E. coli (g/mL)	10	-	-	12	-	-
S. pneumoniae (g/mL)	-	7	-	10	8	-
P. aeruginosa (g/mL)	12	-	-	13	8	-
Ciprofloxacin (mg/mL)	19	16	14	21	19	15

3.3.2 Antimicrobial Inhibition of *A. esculentus* extracts on test organisms

Table 3 reveals that both aqueous and ethanol extracts of *A. esculentus* inhibit all the test organisms except Proteus. Additionally, the ethanol extract showed no antimicrobial activity against *S. pneumoniae.* Both aqueous and ethanol extracts demonstrate microbial growth resistance in a concentration-dependent manner.

Table 3. Antimicrobial Inhibition of *A. esculentus* aqueous and ethanolic extracts

	Zone of Inhibition (mm)					
	Aqueous extract			Ethanolic extract		
Extract Concentration (g/mL)	**0.9**	**0.7**	**0.5**	**0.9**	**0.7**	**0.5**
P. vulgaris (g/mL)	-	-	-	-	-	-
K. pneumoniae (g/mL)	8	-	-	12	8	-
S. aureus (g/mL)	15	13	-	18	15	10
S. typhi (g/mL)	11	-	-	11	10	-
E. coli (g/mL)	-	10	-	17	11	-
S. pneumoniae (g/mL)	11	-	-	-	-	-
Candida (g/mL)	16	-	-	19	17	-
Ciprofloxacin (mg/mL)	23	17	12	25	19	13

In a related study, Sandra (2017) found that aqueous and ethanol extracts of *A. esculentus* seeds were effective against *L. monocytogenes, B. cereus, S. aureus, E. coli*, and *S. enterica* at 100 mg/mL. *L. monocytogenes* was inhibited at all tested concentrations. *S. aureus* was inhibited at 75 and 50 mg/mL, while *B. cereus* and *S. enterica* were not inhibited at these lower concentrations. *E. coli* was also not inhibited at 75 mg/mL, while Bello et al. (2015) reported the ethanolic leaf extract of okra inhibiting antibacterial activities of *Staphylococcus*, *E. coli*, *Klebsiella*, and *Pseudomonas* at various concentrations of 50, 25, 12.5, and 6.25 mg/mL. This supports the result of this study on the susceptibility of the test organisms more to ethanol leaves

extracts over the aqueous extracts. The microbial resistant ability of these plants suggests their possible applications in the food and pharmaceutical industries, in agreement with Carla et al. (2011).

Chaudhari et al. (2011) reported that alcoholic fruit extracts of *A. esculentus* exhibited inhibition of the growth of pathogenic bacteria at a prepared concentration of 50 mcg/mL against *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. pyogenes* in both n-butanol and methanol extracts, while *S. aureus* was only susceptible to the n-butanolic extract. Meanwhile, *P. mirabilis* was not inhibited by both alcoholic extracts. Similarly, Wulandari and Wardani (2019) demonstrated that purple okra fruits inhibits the growth of *E. coli* bacteria. This was shown by inhibition zones, with the most effective concentrations being 40%, 50%, and 60%, which produced zones measuring 6.67 mm. In a research involving the synthesis of nanoparticles from okra pulp, Abdel-Razek et al. (2023) found that gold nanoparticles (Au NPs) of *A. esculentus* pulp aqueous extract (0.2 mg/mL) exhibited antibacterial activity against five tested bacterial strains: *B. subtilis, B. cereus, M. luteus, P. aeruginosa*, and *E. coli*, with inhibition zones of 26, 24, 35, 21, and 15 mm, respectively. Shamsudin et al. (2022), Setiani (2020), Wulandari and Wardani (2019), and Zhou et al. (2023) stated that the antimicrobial potential of *A. esculentus* is due to the presence of phytochemicals responsible for the therapeutic properties of medicinal plants. They noted that flavonoids cause damage to the permeability of bacterial cell walls, microsomes, and lysosomes through interactions between flavonoids and bacterial DNA. Additionally, Solomon et al. (2016) noted that flavonoids are known to exhibit antimicrobial activity through the formation of a complex with the bacterial cell wall.

The antimicrobial activity of both plant solvent extracts is only effective at higher concentrations. Beyond the concentration factor, the type of antimicrobial material produced also determines the ability to inhibit bacterial growth. The antibacterial activity of *A. esculentus* aligns with the report of Wulandari and Wardani (2019), who attributed it to the presence of nutritious compounds such as flavonoids, tannins, saponins, triterpenoids, steroids, and alkaloids.

CONCLUSIONS

The study investigated the antimicrobial activities, phytochemical composition, and thin layer chromatography (TLC) profile of *G. hirsutum* and *A. esculentus* leaf extracts. TLC analysis provided values indicating diverse phytochemical constituents within the extracts. The antimicrobial assays revealed that both plants possess significant antibacterial properties, with ethanolic extracts showing superior efficacy compared to aqueous extracts. These findings underscore the potential of *G. hirsutum* and *A. esculentus* as sources of natural antimicrobial compounds. The superior efficacy of the ethanolic extracts suggests that ethanol is a more effective solvent for extracting bioactive components from these plants. Also, the antimicrobial test findings did not include 0.1 and 0.3 g/mL concentrations since they did not have any action against all the microorganisms tested. This exclusion is therefore not seen as a study limitation but only meant to elaborate on methodology in which they are mentioned in relation to making plant extracts. The preliminary TLC results presented do not fully represent the actual number of compounds in these plant extracts. Recognizing the role of solvent type in the isolation of compounds, future research will explore the use of different solvent mixtures to potentially yield a higher number of spots, indicating the presence of various compounds. This approach aims to enhance the identification of phytochemical constituents in the extracts.

The study offers valuable insights into the phytochemical and antimicrobial properties of these plants. Future studies could focus on isolating specific bioactive compounds responsible for the antimicrobial effects and exploring their mechanisms of action, which could lead to the development of novel therapeutic agents for combating microbial infections.

It should be mentioned that within the TLC analysis, we did not consider using standard references (e.g., rutin and caffeic acid) that are most commonly applied for making comparisons and identification of specific flavonoids and phenolic compounds because this is an inceptive research that aimed at revealing a preliminary profile concerning phytochemicals present in these extracts; therefore, we intend to address this constrained aspect by incorporating standard references into future studies, thus increasing precision during comparison of outcomes."

Conflicts of Interest

The authors declare no personal or financial conflict of interest related to this work.

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